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Kurt T. Smith
Southern Illinois University Carbondale

Gregory Whitledge
Southern Illinois University Carbondale, gwhit@siu.edu

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Trace element and stable isotopic signatures in otoliths and pectoral spines as potential indicators of catfish environmental history

Kurt T. Smith and Gregory W. Whitledge[1]

Fisheries and Illinois Aquaculture Center, Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6511, USA

[1]Corresponding author. Phone: (618) 453-6089. E-mail: gwhit@siu.edu

Running title: Catfish otolith and pectoral spine chemistry

Abstract. - Natural chemical markers in otoliths and fin rays have proven useful for retrospectively describing environmental history of fishes in a variety of environments. However, no studies have applied this technique to catfishes or evaluated catfish pectoral spine chemistry as a non-lethal alternative to otolith chemistry. We characterized relationships between water, otolith, and pectoral spine (articulating process) chemistry for channel catfish *Ictalurus punctatus*, flathead catfish *Pylodictus olivarus*, and blue catfish *Ictalurus furcatus* and determined the accuracy with which fish could be classified to their environment of capture using otolith and pectoral spine chemical signatures. Fish and water samples were collected from nine sites during 2009. Otolith, spine, and water samples were analyzed for Sr:Ca and Ba:Ca; otolith $\delta^{18}O$ and $\delta^{13}C$ and water $\delta^{18}O$ were also measured. Water, otolith, and spine Sr:Ca were highly correlated, as were water and otolith $\delta^{18}O$. Relationships between water, otolith, and spine chemistry did not differ among species. Otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ and spine Sr:Ca differed among sites, reflecting geographic differences in water chemistry. Neither otolith nor spine Ba:Ca differed among sites despite inter-site differences in water Ba:Ca. Both otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ and fin spine Sr:Ca classified fish to their environment of capture with a high degree of accuracy, except in the Middle and Lower Mississippi Rivers where many recent immigrants appeared to be present. Natural chemical signatures in otoliths or pectoral spines will likely be effective for reconstructing environmental history of catfishes when spatial differences in water chemistry are present, enabling investigations of stock mixing and recruitment sources for these species.

Knowledge of environments used by fishes throughout their life history is essential for effective management and conservation of fish populations, especially for riverine species that may move considerable distances for spawning, foraging, or refuge (Fausch et al. 2002). Information regarding environments and habitats that contribute recruits to adult fish stocks as well as fish movement and dispersal patterns are particularly important for understanding metapopulation dynamics (Hanski and Gilpin 1997) and determining the most appropriate spatial scales for managing fisheries. Fish that recruit from local sources and exhibit limited movement are more likely to respond to localized management strategies than are nomadic species (Pugh and Schramm 1999) or stocks that derive recruits from a variety of geographic locations, some of which may be distant from environments occupied by adult fish. Collaborative management efforts of multiple agencies may be required to effectively manage highly mobile species in multi-jurisdictional waters such as the Mississippi River (Pugh and Schramm 1999)

Channel catfish *Ictalurus punctatus*, blue catfish *Ictalurus furcatus*, and flathead catfish *Pylodictus olivarus* are important recreational and commercial fishes whose native ranges overlap in the Mississippi, Mobile, and Rio Grande river drainages (Graham 1999; Hubert 1999; Jackson 1999). Blue catfishes and channel catfishes are particularly mobile species in rivers, with recaptures of tagged individuals often reported > 100 km from the point of release (Graham 1999; Hubert 1999). Blue catfish are predominantly found in large rivers and make long-range movements within these rivers for spawning (Graham 1999); they also inhabit some of the larger tributaries of the Mississippi and Missouri Rivers (Pflieger 1997). Channel catfish are known to migrate into tributaries of large rivers to spawn and feed during spring and summer, returning to large rivers in the fall and winter (Dames et al. 1989; Pellett et al. 1998; Hubert 1999). Flathead catfish also inhabit both large rivers and their tributaries (Pflieger 1997), but are generally more

sedentary than channel catfish or blue catfish, often remaining within 5 km of their release site after tagging (Jackson 1999). However, movements between large river and tributary habitats have been documented for flathead catfish (Dames et al. 1989; Vokoun and Rabeni 2005) and some studies have observed individuals that moved > 50 km (Jackson 1999).

While many telemetry and tagging studies have provided valuable insights into movement and habitat use patterns of older juvenile and adult catfishes (e.g., Pellett et al. 1998; Pugh and Schramm 1999; Vokoun and Rabeni 2005), knowledge of the relative importance of different natal environments and dispersal patterns during early life stages for riverine catfish populations is lacking. Telemetry studies can typically track individual fish for ~ 1 year due to limited transmitter battery life and are restricted to individuals large enough to carry a transmitter (Winter 1996; Vokoun and Rabeni 2005). Relatively low sample sizes in telemetry studies or low recapture rates of fish marked with conventional tags can impede characterization of the diversity and distribution of movement patterns among individuals within a population. Tag loss, non-detection of tags, and potential alteration of tagged fish behavior or growth also represent potential challenges associated with investigating fish movement with conventional tags (Guy et al. 1996). Techniques that could improve our understanding of recruitment sources, early life stage dispersal, and environmental history of individual riverine catfishes throughout their lifetimes would be valuable for conservation and management of these species.

Numerous studies have demonstrated that trace element and stable isotopic compositions of otoliths can serve as natural markers of environmental history for individual fishes in a variety of freshwater environments (Wells et al. 2003; Brazner et al. 2004; Dufour et al. 2005; Munro et al. 2005; Whitledge et al. 2007; Schaffler and Winkelman 2008), including the Middle Mississippi and Illinois Rivers, their tributaries, and floodplain lakes (Whitledge 2009; Zeigler

2009; Zeigler and Whitledge 2010). Otoliths are metabolically inert (Campana and Thorrold 2001) and their Sr:Ca and Ba:Ca ratios and stable isotopic compositions reflect those of environments occupied by a fish (Kennedy et al. 2002; Wells et al. 2003; Dufour et al. 2005; Whitledge et al. 2006; Zeigler and Whitledge 2010). Association of otolith biochronology with isotopic or elemental composition enables retrospective description of fish environmental history when an individual has resided in chemically distinct locations for a period of time sufficient to incorporate the unique signatures of those sites (Kennedy et al. 2002; Dufour et al. 2005; Whitledge et al. 2007). Trace element analysis of sectioned fin rays has been demonstrated to be an effective, non-lethal alternative to otolith chemistry for reconstructing individual fish environmental history in a few freshwater and anadromous fish species (Veinott et al. 1999; Arai et al. 2002; Clarke et al. 2007; Allen et al. 2009). However, we are unaware of any studies that have investigated relationships between water and otolith or pectoral spine chemistry for catfishes or determined whether catfishes from environments with distinct water isotopic or trace elemental signatures can be distinguished using otolith or pectoral spine chemistry.

The goal of this study was to evaluate otolith and pectoral spine chemistry as potential indicators of environmental history for blue catfish, channel catfish, and flathead catfish. Specific objectives were to characterize relationships among water and catfish otolith and pectoral spine chemistry, to test for differences in water-otolith and water-pectoral spine chemistry relationships among these three catfish species, and to determine whether differences in stable isotopic and trace elemental signatures among nine rivers and lakes in the Mississippi River drainage were reflected in catfish otoliths and pectoral spines. We also determined the accuracy with which individual fish could be assigned to the environment in which they were captured using natural chemical signatures in otoliths and pectoral spines. Herein, we also

suggest potential applications of otolith and pectoral spine chemistry as naturally occurring markers of catfish environmental history in the Mississippi River drainage and other areas where spatial differences in water isotopic or trace elemental signatures are present.

Methods

Study Area

Catfishes and water samples were collected from nine sites in the Mississippi River basin (Figure 1). Sampling locations included sites in the Upper Mississippi River (upstream of St. Louis, Missouri), the Middle Mississippi River (between the mouths of the Missouri and Ohio Rivers), the Lower Mississippi River (downstream from the Ohio River confluence), and the Lower Missouri River. Sampling was also conducted in Carlyle Lake, an impoundment on the Kaskaskia River (a tributary of the Middle Mississippi River) in Illinois, the Illinois River, Swan Lake (a floodplain lake connected to the Illinois River near its confluence with the Mississippi River), the Big Muddy River (a Middle Mississippi River tributary), and the Fox River (a tributary of the Illinois River). Sampling locations were chosen because they were known to contain one or more species of catfish and were expected to encompass a broad range of both trace elemental and stable isotopic signatures based on prior studies of water and otolith chemistry for other fish species conducted at these sites (Zeigler 2009; Whitledge 2009; Zeigler and Whitledge 2010).

Water Collection and Analyses

Duplicate water samples for both strontium, barium, and calcium concentrations (for calculation of Sr:Ca and Ba:Ca ratios) and stable oxygen isotopic composition were collected

from each site during summer 2009. Water samples for stable oxygen isotope analysis were collected and stored in scintillation vials containing minimal air space and sealed with Parafilm to curtail evaporative loss and fractionation (Kendall and Caldwell 1998). Water samples were analyzed for stable oxygen isotopic composition using a Thermo Finnigan Delta Plus XL isotope ratio mass spectrometer. All stable isotope ratios were expressed in standard δ notation, defined as the parts per thousand deviation between the isotope ratio of a sample and standard material (Vienna Standard Mean Ocean Water for water $\delta^{18}O$):

$$\delta^{18}O\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000;$$

where R represents $^{18}O/^{16}O$. Mean standard deviation of replicate measurements of water $\delta^{18}O$ was 0.3‰ (n = 4 replicates per sample). Water samples for analysis of strontium, barium, and calcium concentrations were collected using a syringe filtration technique described in Shiller (2003). Samples for analysis of elemental concentrations were stored on ice or refrigerated until overnight shipment and analysis by high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS). Elemental concentration data were converted to molar Sr:Ca and Ba:Ca ratios (mmol/mol). Mean standard deviations of replicate measurements of water Sr:Ca and Ba:Ca were 0.03 and 0.02 mmol/mol, respectively.

Otolith and Pectoral Fin Spine Preparation and Analyses

Blue catfish, channel catfish, and flathead catfish were collected during summer 2009 using hoop nets, trap nets, boat electrofishing, or angling. Blue catfish (205-300 mm total length (TL), 66-210 g wet weight) were collected from the Missouri, Middle Mississippi, and Big Muddy Rivers. Channel catfish (232-620 mm TL, 113-2,430 g wet weight) were collected at all sites except for the Missouri and Middle Mississippi Rivers. Flathead catfish (135-510 mm TL,

26-1,394 g wet weight) were collected from all sites. Catfishes were euthanized with MS-222, placed on ice for transport to the laboratory, and stored frozen until otolith and pectoral spine removal.

Lapilli otoliths were obtained from each fish by sectioning through the supraoccipital bone anterior to the base of the pectoral fins using the center of the fontanelles (soft indentations on top of the head) as a landmark (Nash and Irwin 1999; Buckmeier 2002). Otoliths were removed using non-metallic forceps, cleaned with distilled water, and stored dry in polyethylene microcentrifuge tubes until preparation for stable isotope and trace element analyses. One otolith from each fish (with the exception of fish from the Fox River) was analyzed for stable oxygen and carbon isotopic compositions. A subsample chipped from the outer edge of otoliths > 1 mg was pulverized using acid washed mortar and pestle, as this portion of the otolith reflects a fish's most recent environmental history. Otoliths < 1 mg were pulverized whole for stable isotope analysis. Pulverized otolith samples were analyzed for stable oxygen and carbon isotopic compositions using a ThermoFinnigan Delta plus XP isotope ratio mass spectrometer interfaced with a Gas Bench II carbonate analyzer. Stable oxygen and carbon isotope ratios for otolith samples were expressed in standard δ notation ($\delta^{18}O$ or $\delta^{13}C$, ‰; parts per thousand deviation between the isotope of a sample and standard Pee Dee Belemnite for $\delta^{18}O$ or $\delta^{13}C$ in otolith carbonate); mean standard deviation for replicate measurements (n = 2 per sample) was 0.06‰ for $\delta^{18}O$ and 0.04‰ for $\delta^{13}C$.

One pectoral spine and the second otolith from each fish were used for trace element analysis. Pectoral spines were removed, embedded in epoxy, and sectioned at the articulating process (the widest portion at the base of the spine) using a Buehler ISOMET low-speed saw (Turner 1982; Buckmeier 2002). Otoliths were embedded in epoxy and cut into 1.3 mm sections

surrounding the nucleus in the transverse plane using an ISOMET low-speed saw. Otolith and pectoral spine sections were sanded and polished to reveal annuli, mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under a class 100 laminar flow hood. Mounted and cleaned otolith and spine sections were stored in acid-washed polypropylene Petri dishes in a sealed container until analysis. Otolith and pectoral spine sections were analyzed for ^{88}Sr, ^{137}Ba, and ^{44}Ca using a Perkin-Elmer DRC II inductively coupled plasma mass spectrometer (ICPMS) coupled with a CETAC Technologies LSX-500 laser ablation system. The laser ablated a transect from the core to the edge of each sectioned otolith and pectoral spine along the longest axis (beam diameter = 50 µm, scan rate = 10 µm/s, laser pulse rate = 10 Hz, laser energy level = 70%, wavelength = 213 nm). A standard developed by the U. S. Geological Survey (MACS-1, $CaCO_3$ matrix) was analyzed every 12-15 samples to adjust for possible instrument drift. Each sample analysis was preceded by a gas blank measurement. Isotopic counts were converted to elemental concentrations (µg/g) after correction for gas blank, matrix, and drift effects. Mean limits of detection for ^{88}Sr and ^{137}Ba were 0.06 and 0.35 µg/g, respectively; concentrations of these elements in all otoliths and pectoral spines were well above detection limits. Otolith and pectoral spine Sr and Ba concentrations were calculated from integrations over the final 50 µm of laser ablation transects, as the outer portions of these structures reflect a fish's most recent environmental history. Strontium and barium concentrations were normalized to calcium (Ca) concentration based on the consideration of Ca as a pseudointernal standard (Ludsin et al. 2006); data are reported as Sr:Ca and Ba:Ca ratios (mmol/mol).

Statistical Analyses

One-way analyses of variance (ANOVAs) followed by Tukey's HSD tests for multiple comparisons were used to test for significant differences in individual water chemistry parameters ($\delta^{18}O$, Sr:Ca, and Ba:Ca) among sampling locations. Differences in relationships between water and otolith Sr:Ca, Ba:Ca, and $\delta^{18}O$ and between water and pectoral spine Sr:Ca and Ba:Ca among the three catfish species were assessed with analyses of covariance (ANCOVAs) with water Sr:Ca, Ba:Ca, or $\delta^{18}O$ as the covariate. Linear regressions were used to characterize relationships between water and otolith Sr:Ca, Ba:Ca, and $\delta^{18}O$, water and pectoral spine Sr:Ca and Ba:Ca, and otolith and pectoral spine Sr:Ca and Ba:Ca for individual fish. The 95% confidence intervals for the slopes of individual regressions were used to determine whether the slope differed from one when linear regressions indicated significance between otolith and pectoral spine Sr:Ca and Ba:Ca for individual fish.

Both univariate and multivariate approaches were employed to evaluate and describe differences in catfish otolith and pectoral spine chemical signatures among sites. One-way ANOVAs followed by Tukey's HSD tests were used to test for significant differences in means for otolith Sr:Ca, Ba:Ca, $\delta^{18}O$, and $\delta^{13}C$ and pectoral spine Sr:Ca and Ba:Ca among sampling locations. Individual otolith chemistry parameters that differed significantly among sampling locations in conjunction with inter-site differences in water chemistry were entered into a multivariate analysis of variance (MANOVA) and a discriminant analysis (CANDISC procedure in SAS) to characterize the multivariate otolith chemistry signatures of catfish from rivers and lakes sampled in this study; a plot of the first two canonical variates was used to visually depict differences in catfish otolith chemistry signatures among sites. Significance of differences in multivariate otolith chemistry signatures among sites was assessed using Pillai's trace statistic.

Linear discriminant function analysis (LDFA) with a leave-one-out jackknife procedure was used to determine the accuracy with which individual fish could be classified back to their collection location based on their otolith elemental and stable isotopic signatures. Two additional LDFAs were performed to determine classification accuracy for individual fish to environments with high (> 3 mmol/mol), moderate (2.25-2.5 mmol/mol), and low (1.29-1.63 mmol/mol) mean water Sr:Ca values using otolith Sr:Ca or pectoral spine Sr:Ca values. P-values ≤ 0.05 were considered significant for all statistical tests.

Results

Differences in water chemistry among sampling sites

Mean water Sr:Ca differed among rivers and lakes sampled during this study ($P < 0.0001$), with the highest water Sr:Ca occurring in the Fox River (Figure 2). Water Sr:Ca was higher in the Missouri River compared to all other sites except the Fox River. The Big Muddy River and Lower and Middle Mississippi Rivers had intermediate water Sr:Ca values, while the lowest water Sr:Ca values were observed in Carlyle and Swan Lakes and the Illinois and Upper Mississippi Rivers. Mean water Ba:Ca also differed among our sampling sites ($P < 0.0001$; Figure 2). Water Ba:Ca was significantly higher in the Missouri River compared to all other sites except Carlyle Lake. The lowest water Ba:Ca values were observed in the Big Muddy, Illinois, and Lower Mississippi Rivers and Swan Lake. Mean water $\delta^{18}O$ also differed among sampling sites ($P < 0.0001$; Figure 2). The Missouri and Middle Mississippi Rivers exhibited the most negative water $\delta^{18}O$ values, although water $\delta^{18}O$ for these rivers were not significantly different from that of the Upper Mississippi River. The Fox, Illinois, and Lower Mississippi

Rivers had intermediate water $\delta^{18}O$ values, while the least negative $\delta^{18}O$ values were observed in Swan Lake, the Big Muddy River, and Carlyle Lake.

Differences in otolith and pectoral spine chemistry among species

No significant differences among the three catfish species were observed for relationships between water and otolith edge Sr:Ca ($P = 0.76$), water and pectoral spine edge Sr:Ca ($P = 0.11$), water and otolith edge Ba:Ca ($P = 0.48$), or water and pectoral spine edge Ba:Ca ($P = 0.27$). Similarly, no significant differences in relationships between water and otolith edge $\delta^{18}O$ among species were observed ($P = 0.12$). Therefore, otolith and pectoral spine chemistry data from the three species of catfish were pooled in subsequent analyses.

Relationships among water, otolith, and pectoral spine chemistry

Both pectoral spine edge Sr:Ca and otolith edge Sr:Ca were strongly correlated with water Sr:Ca ($r^2 = 0.68$, $P < 0.0001$; $r^2 = 0.75$, $P < 0.0001$, respectively; Figure 3). Otolith edge $\delta^{18}O$ was also correlated with water $\delta^{18}O$ ($r^2 = 0.42$, $P < 0.0001$; Figure 4). However, neither pectoral spine edge Ba:Ca ($P = 0.09$) nor otolith edge Ba:Ca ($P = 0.50$) were correlated with water Ba:Ca. Pectoral spine edge Sr:Ca and otolith edge Sr:Ca for individual fish were strongly correlated ($r^2 = 0.58$, $P < 0.0001$; Figure 5). The slope of the regression line (0.30 ± 0.03 SE) relating pectoral spine and otolith Sr:Ca was significantly less than one ($P < 0.05$). Pectoral spine edge Ba:Ca and otolith edge Ba:Ca for individual fish were not correlated ($P = 0.85$).

Differences in otolith and pectoral spine chemistry among sampling locations

Mean pectoral spine edge Sr:Ca was significantly different among sampling locations ($P < 0.0001$) and reflected trends observed for water Sr:Ca (Figure 6a). The highest mean pectoral spine edge Sr:Ca values were observed in fish from the Fox and Missouri Rivers. Fish from the Illinois River, Swan Lake, and Carlyle Lake had the lowest mean pectoral spine edge Sr:Ca values. Mean otolith edge Sr:Ca also differed among sites ($P < 0.0001$; Figure 6b). Mean otolith edge Sr:Ca was highest in fish from the Fox River, intermediate in fish collected from the Missouri River, and lowest at the other seven sites. Mean otolith edge $\delta^{18}O$ for catfishes was significantly different among sampling locations ($P < 0.0001$; Figure 7a). With the exception of fish collected in the Middle Mississippi River, differences in otolith edge $\delta^{18}O$ among sites generally reflected inter-site differences in water $\delta^{18}O$. Mean otolith edge $\delta^{13}C$ differed among fish from different sampling locations ($P < 0.0001$, Figure 7b). Fish from the Missouri River had less negative otolith edge $\delta^{13}C$ values compared to fish collected from all other sites except Swan Lake. However, otolith edge $\delta^{13}C$ values did not differ among the other sites. Neither mean pectoral spine edge Ba:Ca nor mean otolith edge Ba:Ca differed among sites ($P = 0.10$ and $P = 0.68$, respectively).

Multivariate analysis of otolith data incorporating $\delta^{18}O$, $\delta^{13}C$, and Sr:Ca indicated that fish sampled from different sites possessed significantly different otolith chemistry signatures (Pillai's trace statistic, $P < 0.0001$). A plot of the first two canonical variates from the CANDISC procedure in SAS illustrated these distinct otolith chemistry signatures among sites (Figure 8). The first two discriminant functions (CAN1 and CAN2) from this model accounted for 96% of the total dispersion in the dataset. With the exception of fish collected in the Middle Mississippi and Lower Mississippi Rivers, individual fish captured within a particular river or lake possessed similar multivariate otolith chemistry signatures (Figure 8).

Classification accuracy of individual fish to environment of capture using otolith and fin spine chemistry

Linear discriminant function analysis with a leave-one-out jackknife procedure indicated that classification success for individual fish to their location of capture based on otolith edge Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ was relatively poor (43-60% accuracy) for fish collected in the Middle and Lower Mississippi Rivers (Table 1). Classification success to environment of capture was higher (71-100% accuracy) for individuals collected from other rivers and lakes. Misclassification rates to site of capture were 18% for both channel catfish and flathead catfish and 20% for blue catfish. Results of the second LDFA indicated that individual catfish could be assigned to the type of environment in which they were collected (high, moderate, or low water Sr:Ca) with 67-87% accuracy based on pectoral spine edge Sr:Ca and with 87-100% accuracy using otolith edge Sr:Ca (Table 2).

Discussion

Catfish otolith edge Sr:Ca and $\delta^{18}O$ and pectoral spine edge Sr:Ca were strongly correlated with corresponding water Sr:Ca and $\delta^{18}O$ values of rivers and lakes in the Mississippi River basin, consistent with published studies that have also reported highly significant linear relationships between water and otolith Sr:Ca and $\delta^{18}O$ or water and fin ray Sr:Ca for other freshwater fish species (Wells et al. 2003; Clarke et al. 2007; Walther and Thorrold 2008; Zeigler and Whitledge 2010). With the exception of several individuals collected in the Middle and Lower Mississippi Rivers, differences in catfish otolith edge Sr:Ca, $\delta^{18}O$ and pectoral spine edge Sr:Ca among sampling locations reflected differences in water Sr:Ca and $\delta^{18}O$ sites.

Differences in water Sr:Ca and $\delta^{18}O$ and catfish otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ among rivers and lakes sampled in this study were consistent with geographic differences in water and otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ observed in previous studies with other fish species (centrarchids, moronids, and freshwater drum *Aplodinotus grunniens*) in the same rivers and lakes (Whitledge 2009; Zeigler 2009; Zeigler and Whitledge 2010). The absence of significant differences in catfish otolith or pectoral spine Ba:Ca among our sampling sites despite the presence of inter-site differences in water Ba:Ca and the lack of significant relationships between otolith and pectoral spine Ba:Ca and water Ba:Ca was surprising considering that other studies have found otolith or fin ray Ba:Ca to be a useful natural marker of environmental history for freshwater fishes in other locations (Wells et al. 2003; Brazner et al. 2004; Ludsin et al. 2006; Clarke et al. 2007). However, Ba:Ca in otoliths, fin rays, or scales has previously been shown to be less strongly correlated with water Ba:Ca in comparison to correlations between Sr:Ca in water and Sr:Ca in hard structures of freshwater fishes (Wells et al. 2003; Clarke et al. 2007; Zeigler and Whitledge 2010). Otolith Ba:Ca was also less effective at discriminating among fishes (centrarchids, moronids, and freshwater drum) from the Mississippi and Illinois Rivers, their tributaries, and floodplain lakes in comparison to otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$ (Zeigler 2009; Zeigler and Whitledge 2010).

Classification success rates for individual catfish to environment of capture in this study were comparable to or greater than those of published studies using otolith or fin ray microchemistry or stable isotopic compositions as indicators of source location for freshwater fishes (Bronte et al. 1996; Wells et al. 2003; Brazner et al. 2004; Clarke et al. 2007; Schaffler and Winkelman 2008; Whitledge 2009; Zeigler and Whitledge 2010). Misclassifications of individual fish to environment of capture were likely due to the presence of recent immigrants in

some rivers and inclusion of sites that possessed water chemistry signatures that were indistinguishable from one another. All fish from the Illinois River whose environment of capture was not correctly identified were classified as having come from the Upper Mississippi River and all but one of the misclassified individuals from the Upper Mississippi River were identified as Illinois River fish. These "errors" are not surprising given the indistinguishable water chemistry signatures of these two sites. Other classification errors for individual fish to environment of capture occurred among fish collected in the Middle Mississippi and Lower Mississippi Rivers, with two individuals from the Big Muddy River also misidentified as having come from the Middle Mississippi River. It is likely that the two misclassified individuals captured from the Big Muddy River were recent immigrants from the nearby Middle Mississippi River that had not been in the Big Muddy River long enough to acquire the chemical signature of the tributary in their otoliths or pectoral spines. Blue catfish, channel catfish, and flathead catfishes are known to be mobile in riverine environments, including movement among river reaches and between rivers and tributaries (Dames et al. 1989; Pellett et al. 1998; Graham 1999; Hubert 1999; Pugh and Schramm 1999; Travnichek 2004; Vokoun and Rabeni 2005). Further research should investigate the relative importance of different environments as recruitment sources for catfishes in the Mississippi River and tributaries.

The applicability of otolith, fin ray, or fin spine chemistry as a natural indicator of fish environmental history across years in a given location depends on the persistence of geographically-based differences in water chemistry signatures over time. Differences in water Sr:Ca and $\delta^{18}O$ among rivers and lakes sampled in this study were consistent with spatial differences in water Sr:Ca and $\delta^{18}O$ documented by studies in this portion of the Mississippi River drainage that were conducted during 2006-2007 (Whitledge 2009; Zeigler 2009; Zeigler

and Whitledge 2010). Mean water $\delta^{18}O$ values for the middle Mississippi, lower Missouri, Illinois, and Big Muddy Rivers during this study were also within the ranges of water $\delta^{18}O$ values reported for these rivers by Coplen and Kendall (2000), suggesting that $\delta^{18}O$ signatures of these rivers exhibit some stability across years. Substantial temporal variation in environmental signatures could limit the utility of catfish otolith and pectoral spine chemical signatures as tracers of fish environmental history during some years or may require a ‘‘library’’ of environmental signatures and separate classification models for identifying natal environments of fish from different year classes (Ludsin et al. 2006; Schaffler and Winkelman 2008).

We found no significant differences in relationships between water and otolith Sr:Ca, Ba:Ca, or $\delta^{18}O$ or water and pectoral spine Sr:Ca or Ba:Ca among the three catfish species sampled. Other studies have also reported no differences in relationships between water and otolith Sr:Ca or $\delta^{18}O$ among closely related freshwater fish species (Patterson et al. 1993; Whitledge et al. 2007; Zeigler and Whitledge 2010). However, Zeigler and Whitledge (2010) found that freshwater drum exhibited elevated otolith Ba:Ca values compared to several species of centrarchids collected from the same water bodies. Species-specific incorporation of trace elements (Mg, Mn, Sr, and Ba) into otoliths has also been detected in some saltwater fishes (Swearer et al. 2003; Hamer and Jenkins 2007). While the results of our study suggest that relationships between water and otolith or pectoral spine Sr:Ca, Ba:Ca, and $\delta^{18}O$ are consistent among blue catfish, channel catfish, and flathead catfish, these relationships between water and fish structure chemistry should not be assumed to be applicable to other catfish species.

The highly significant linear relationship between Sr:Ca in water and the articulating process of catfish pectoral spines and significant differences in mean pectoral spine edge Sr:Ca among rivers and lakes that mirrored inter-site differences in water and catfish otolith edge Sr:Ca

suggest that measurements of pectoral spine articulating process Sr:Ca may represent a non-lethal alternative to otolith Sr:Ca as a natural marker of catfish environmental history. Strong correlations between fin ray and water Sr:Ca have been documented in other freshwater and anadromous fish species (Veinott et al. 1999; Arai et al. 2002; Clarke et al. 2007; Allen et al. 2009); Clarke et al. (2007) found that the relationship between water and fin ray Sr:Ca was stronger than that of water Sr:Ca and otolith Sr:Ca for Arctic grayling *Thymallus arcticus*. Pectoral spine Sr:Ca was effective for distinguishing among catfishes from sites with high (> 3 mmol/mol), moderate (2.25-2.5 mmol/mol) and low (1.29-1.63 mmol/mol) mean water Sr:Ca. However, classification accuracy of individual fish to collection sites that differed in water Sr:Ca was generally higher for otolith Sr:Ca than pectoral spine Sr:Ca. Both pectoral spines and otoliths have been used to age channel, blue, and flathead catfishes (Turner 1982; Holland-Bartels and Duval 1988; Graham 1999; Nash and Irwin 1999; Buckmeier et al. 2002). Otoliths and the pectoral spine articulating process provide greater accuracy and precision for estimating age of catfishes compared to basal recess sections of pectoral spines (Turner 1982; Nash and Irwin 1999), as loss of early annuli can occur in basal recess sections of older fish due to expansion of the central lumen (Nash and Irwin 1999). The presence of the central lumen also makes basal recess sections unsuitable for applications of pectoral spine chemistry to identify natal origin or reconstruct environmental history of catfishes. Therefore, articulating process sections are recommended for applications of catfish pectoral spine chemistry. When compared to otoliths, pectoral spines sectioned at the articulating process underestimated ages of flathead catfish > age 15 (Nash and Irwin 1999); otoliths also provided greater accuracy than pectoral spines for aging age 1-4 channel catfish (Buckmeier et al. 2002). Thus, while either otoliths or pectoral spine articulating process sections will likely be suitable for identifying natal

environment or retrospectively describing movement patterns during larval and juvenile life stages for juvenile or adult catfishes using natural chemical signatures in these structures, we suggest that otoliths may be superior to articulating process sections for reconstructing timing of adult catfish movements among chemically distinct environments when sacrificing fish for otolith removal is not a concern.

Potential applications of catfish otolith or pectoral spine chemistry as natural environmental markers in the Mississippi River basin and in other areas where geographic differences in water chemistry exist include determination of the relative importance of different environments as recruitment sources for catfish populations, investigations of stock mixing, and characterization of movement and dispersal patterns, particularly during early life stages. While many telemetry and tagging studies have provided valuable insights into movement and habitat use patterns of older juvenile and adult catfishes (e.g., Pellett et al. 1999; Pugh and Schramm 1999; Vokoun and Rabeni 2005), much less is known about natal environments and dispersal of catfishes during early life stages. We anticipate that application of otolith and pectoral spine chemistry will provide new knowledge of catfish early life environmental history, which is difficult to investigate using other methods. Knowledge of which environments represent important natal or larval and juvenile nursery areas is potentially important for informing efforts to maintain or restore habitats that contribute to catfish recruitment. Investigations of stock mixing using otolith or pectoral spine chemistry could provide additional insight into the spatial scale at which catfish populations and fisheries should be managed, including the need for inter-jurisdictional, cooperative management. Otolith or pectoral spine chemistry may also enable identification of source environment for fish illegally introduced into areas outside of their native range, an application of otolith chemistry that has previously been demonstrated for salmonids

(Munro et al. 2005). Fish hard part chemistry can also differentiate stocked from wild fish when hatchery and stocking sites possess distinct chemical signatures (Bickford and Hannigan 2005; Gibson-Reinemer et al. 2009), enabling evaluation of stocking success using natural tags; we anticipate that this approach will also be applicable to catfishes reared in hatcheries with naturally distinct water chemistry signatures.

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Table 1. Results of linear discriminant function analysis showing classification accuracy (determined by jackknife procedure) for individual fish to the environment in which they were captured based on otolith Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$.

Source location	n	% correct
Big Muddy River	7	71
Carlyle Lake	11	100
Illinois River	8	88
Lower Mississippi River	10	60
Middle Mississippi River	7	43
Missouri River	12	100
Upper Mississippi River	14	71

Table 2. Results of linear discriminant function analysis showing classification accuracy (determined by jackknife procedure) for individual fish to their environment of capture (high, moderate, or low water Sr:Ca) based on pectoral spine edge Sr:Ca or otolith Sr:Ca. Sites with high water Sr:Ca included the Fox and Missouri Rivers, sites with moderate water Sr:Ca included the Big Muddy, Lower Mississippi, and Middle Mississippi Rivers, and sites with low water Sr:Ca were represented by Carlyle Lake, the Illinois River, Swan Lake, and the Upper Mississippi River.

Source location water Sr:Ca	n	% correct (spines)	% correct (otoliths)
High (> 3 mmol/mol)	16	81	100
Moderate (2.25-2.5 mmol/mol)	30	67	88
Low (1.29-1.63 mmol/mol)	39	87	87

Figure Captions

Figure 1. Map of the Mississippi River drainage in Illinois and Missouri showing locations (filled diamonds) where catfishes and water samples were collected.

Figure 2. Mean water Sr:Ca, Ba:Ca, and $\delta^{18}O$ values (± SE) for rivers and lakes sampled during this study. Within each panel, means marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $P > 0.05$).

Figure 3. Relationships between (a) catfish pectoral spine edge Sr:Ca and water Sr:Ca ($y = 0.146 x + 0.149$) and (b) catfish otolith edge Sr:Ca and water Sr:Ca ($Y = 0.263 x – 0.076$). Solid lines are least-squares linear regression functions fit to data.

Figure 4. Relationship between catfish otolith $\delta^{18}O$ and water $\delta^{18}O$. Solid line is the least-squares linear regression function fit to data ($y = 0.624 x – 4.163$).

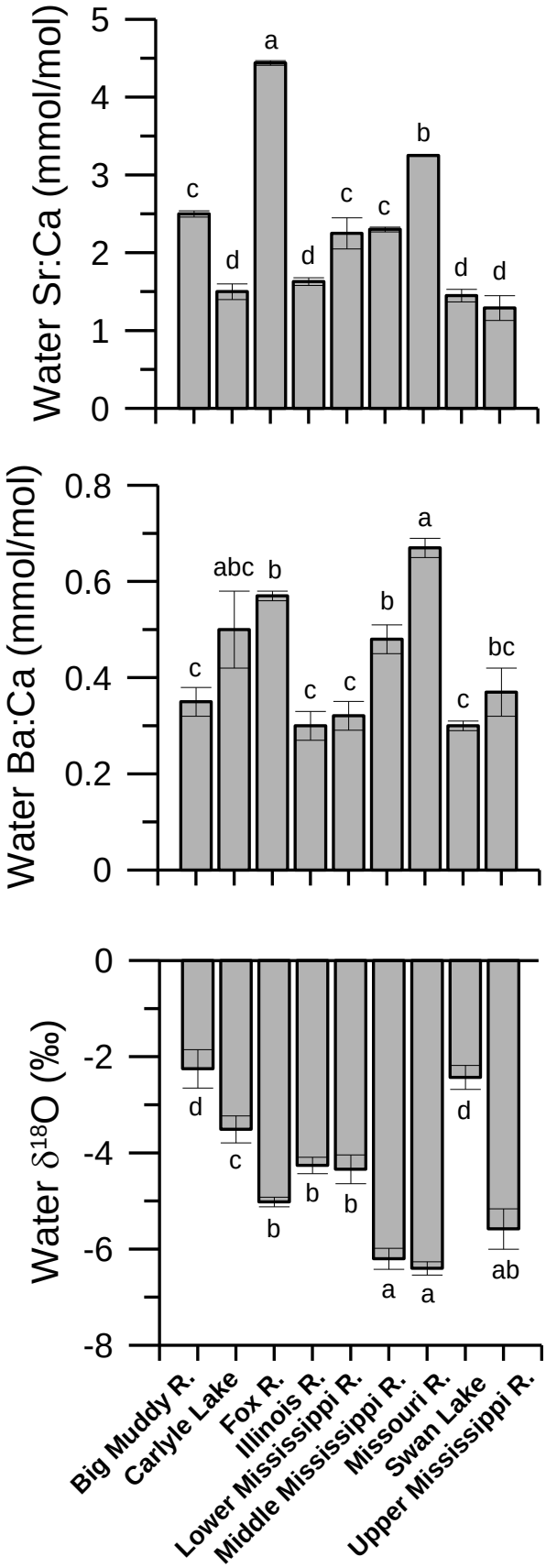
Figure 5. Relationship between catfish pectoral spine edge Sr:Ca and catfish otolith edge Sr:Ca. The dashed line is the line of 1:1 correspondence and the solid line is the least-squares linear regression function fit to data ($y = 0.529 x – 0.198$).

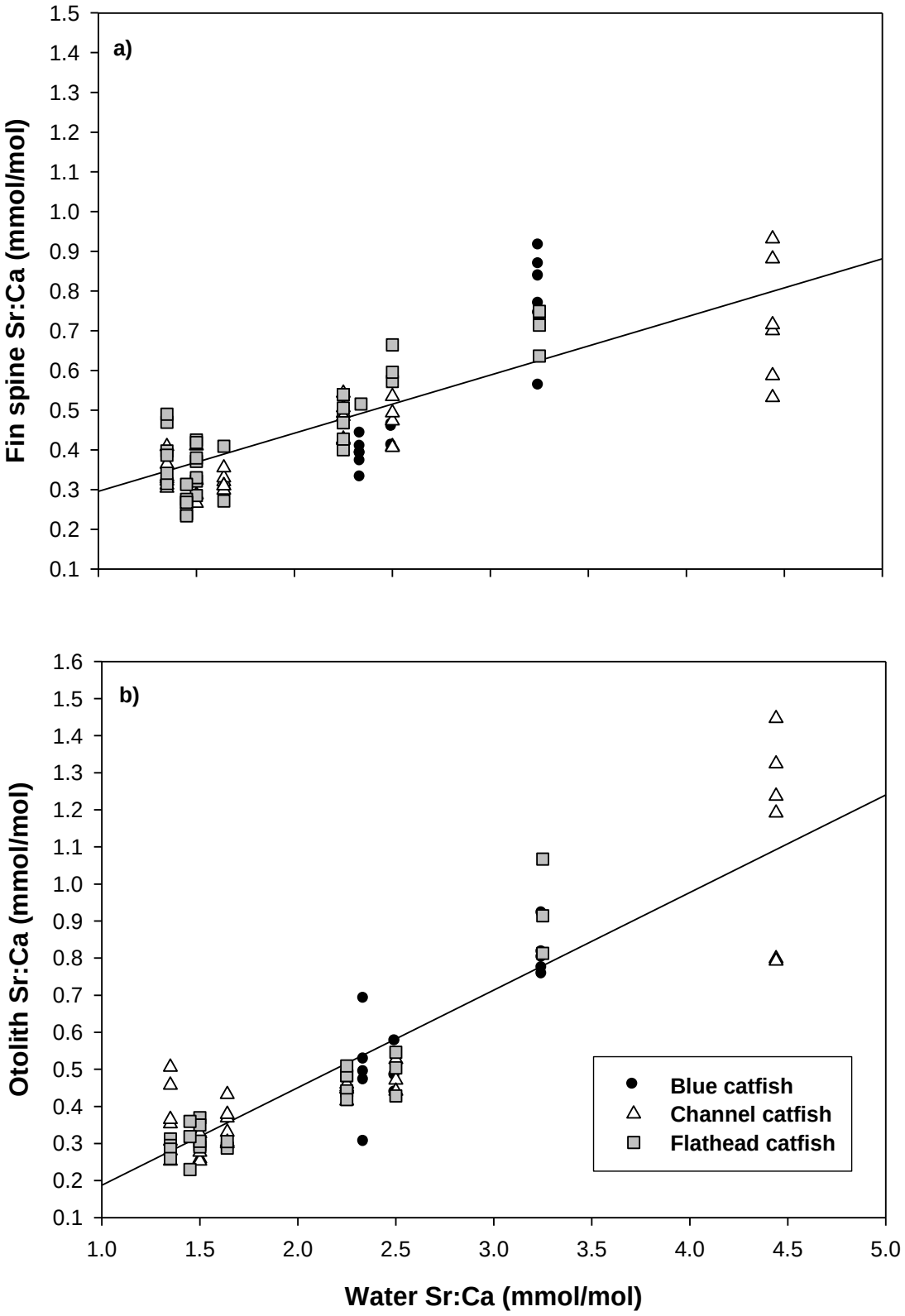
Figure 6. (a) Mean catfish pectoral spine edge Sr:Ca (± SE) and (b) mean catfish otolith edge Sr:Ca (± SE) for each of the rivers and lakes sampled during this study. Within each panel, means marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $P > 0.05$).

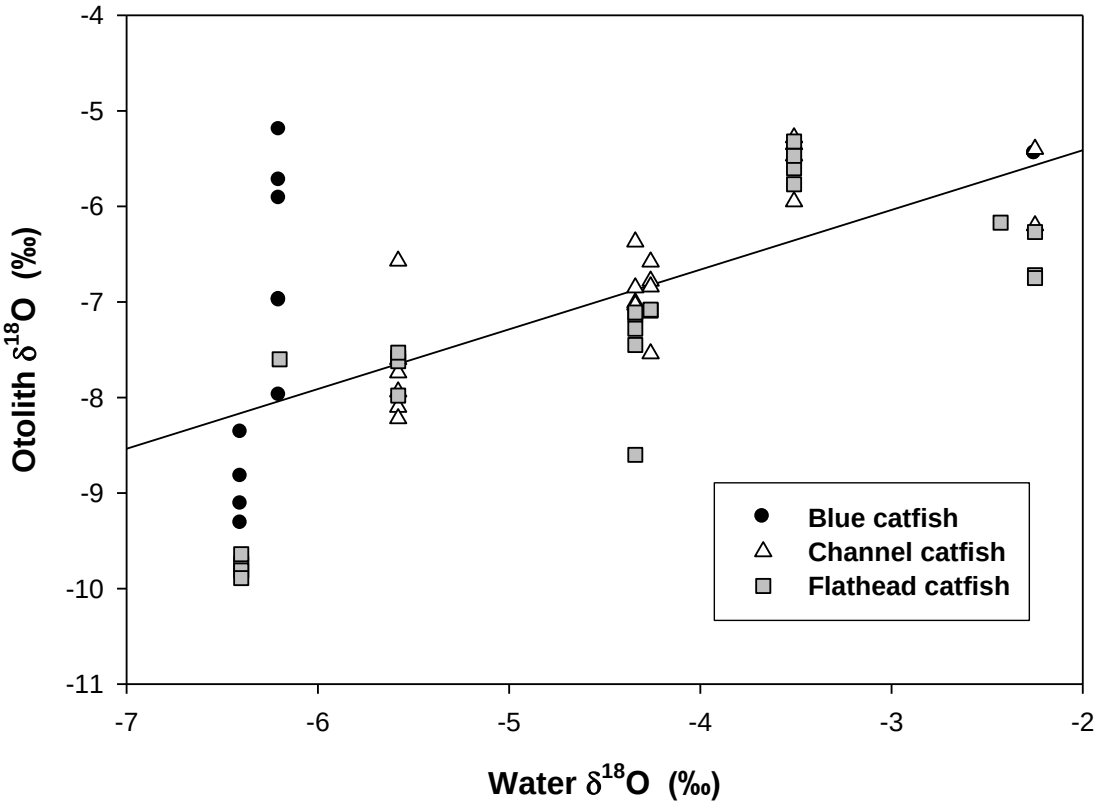
Figure 7. (a) Mean catfish otolith edge $\delta^{18}O$ (± SE) and (b) mean catfish otolith edge $\delta^{13}C$ (± SE) for each of the rivers and lakes sampled during this study. Within each panel, means marked with the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $P > 0.05$).

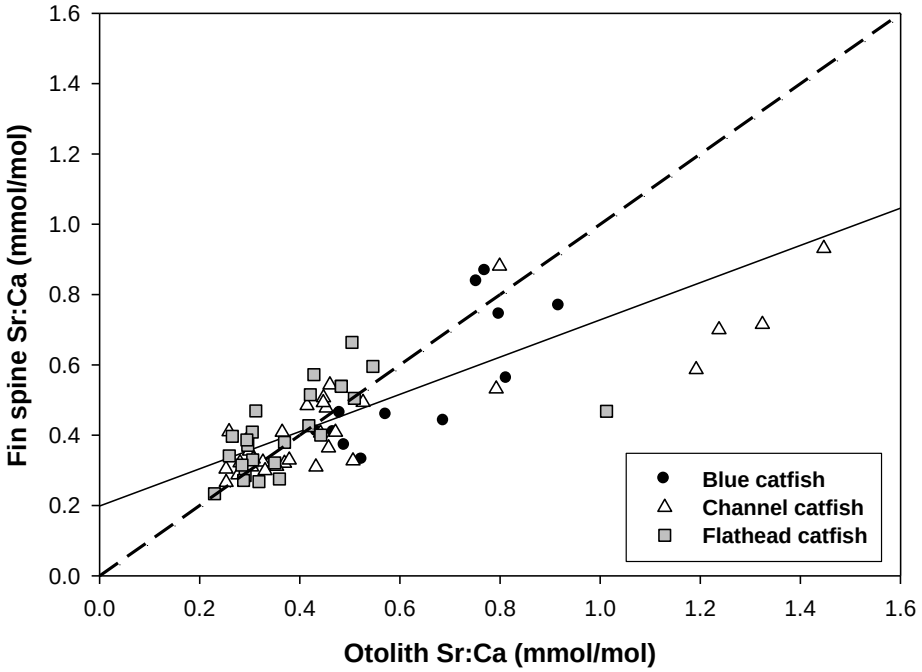
Figure 8. Multivariate otolith chemistry signatures for catfishes collected from seven locations in the middle portion of the Mississippi River basin as represented by the first two canonical variates obtained through linear discriminant function analysis including otolith edge Sr:Ca, $\delta^{18}O$, and $\delta^{13}C$.

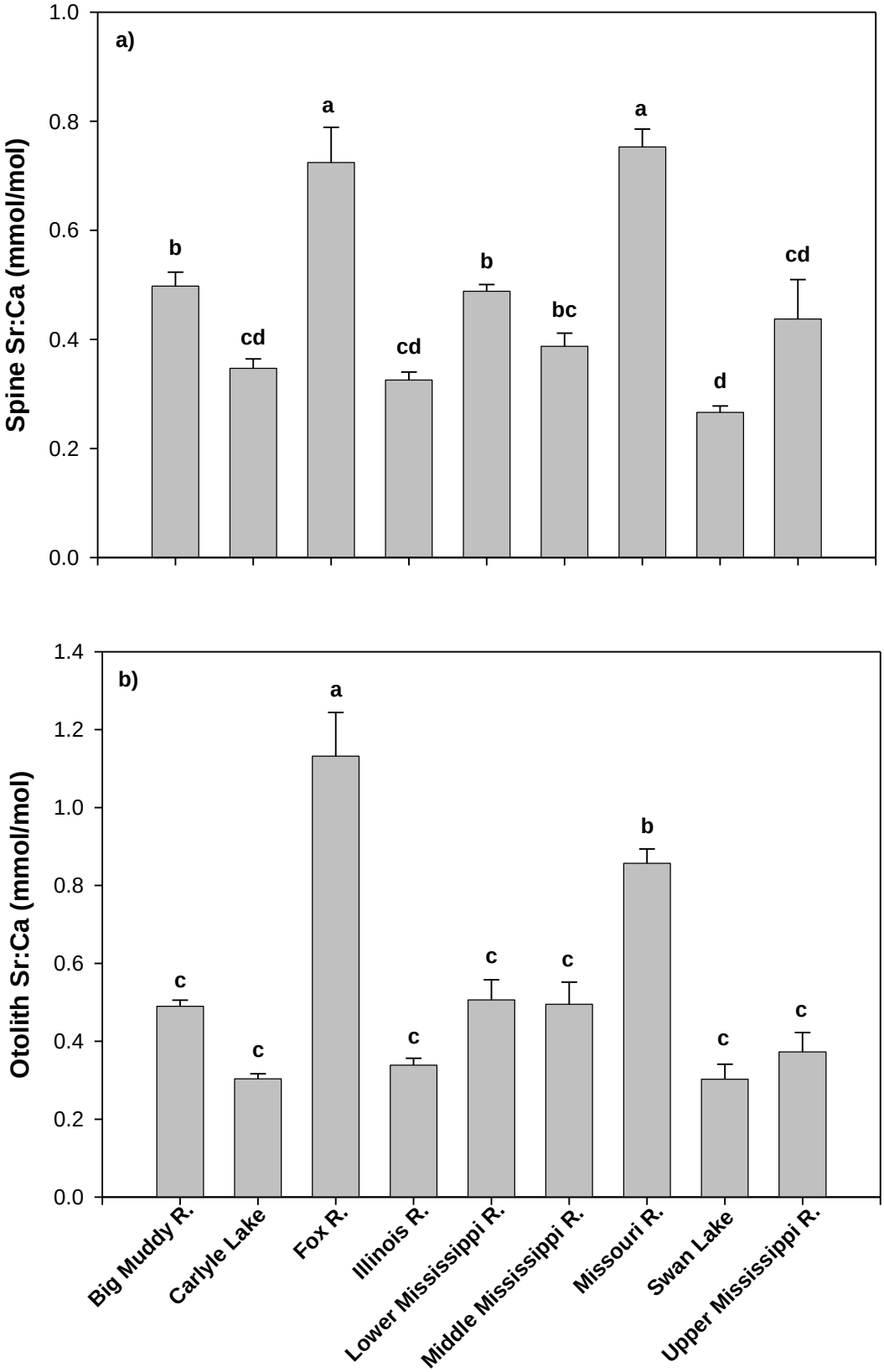

Fox
River
ILLINOIS
Upper
Mississippi
River
Illinois
River
Swan
Lake
Carlyle
Lake
Missouri River
Big
Muddy
River
MISSOURI
Middle
Mississippi
River
Lower
Mississippi
River

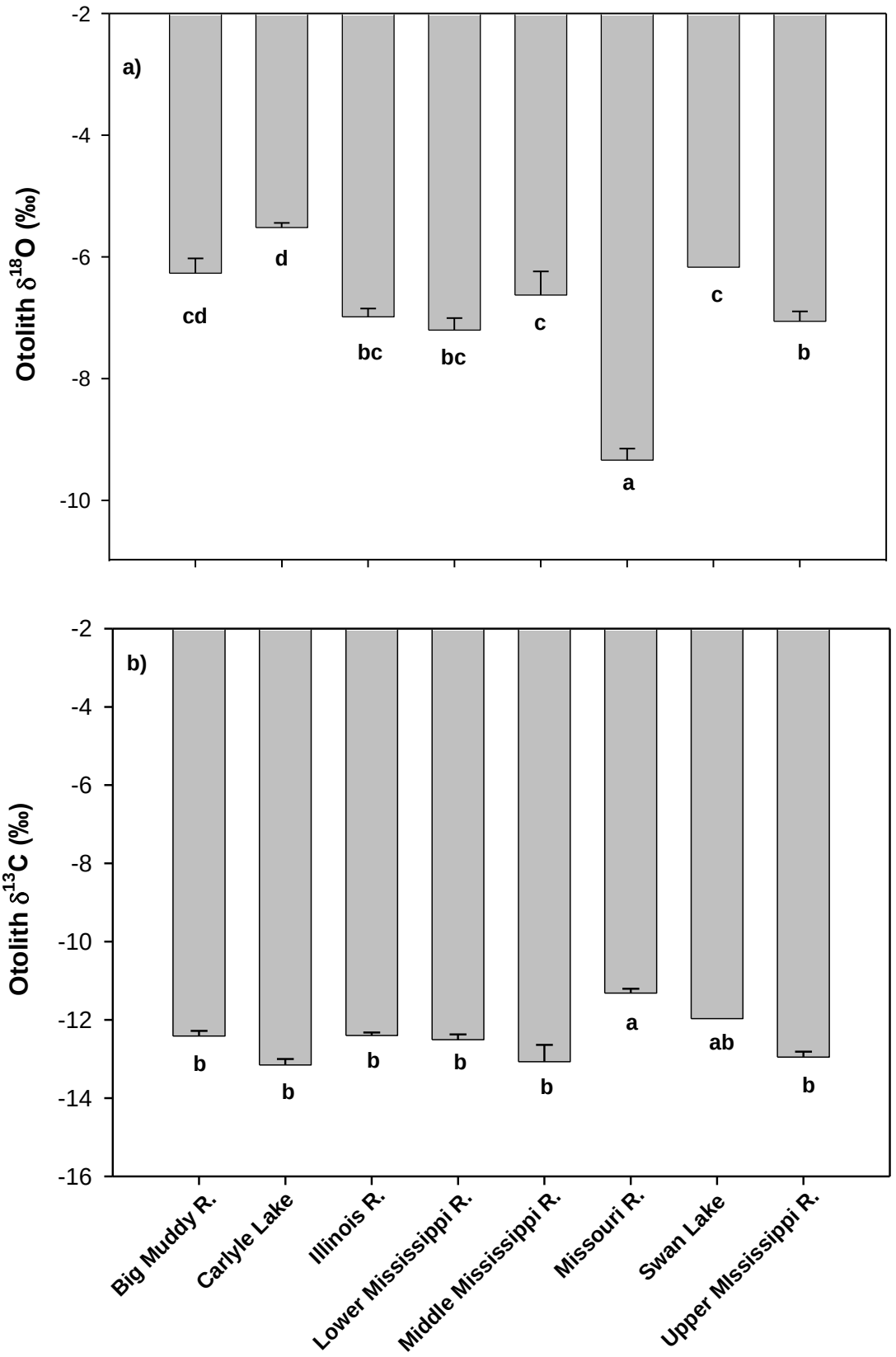

Water Sr:Ca (mmol/mol)
0
1
2
3
4
5
c
d
a
d
c
c
b
d
d
Water Ba:Ca (mmol/mol)
0
0.2
0.4
0.6
0.8
c
abc
b
c
c
b
a
c
bc
Water δ18O (‰)
0
-2
-4
-6
-8
d
c
b
b
b
a
a
d
ab
Big Muddy R.
Carlyle Lake
Fox R.
Illinois R.
Lower Mississippi R.
Middle Mississippi R.
Missouri R.
Swan Lake
Upper Mississippi R.

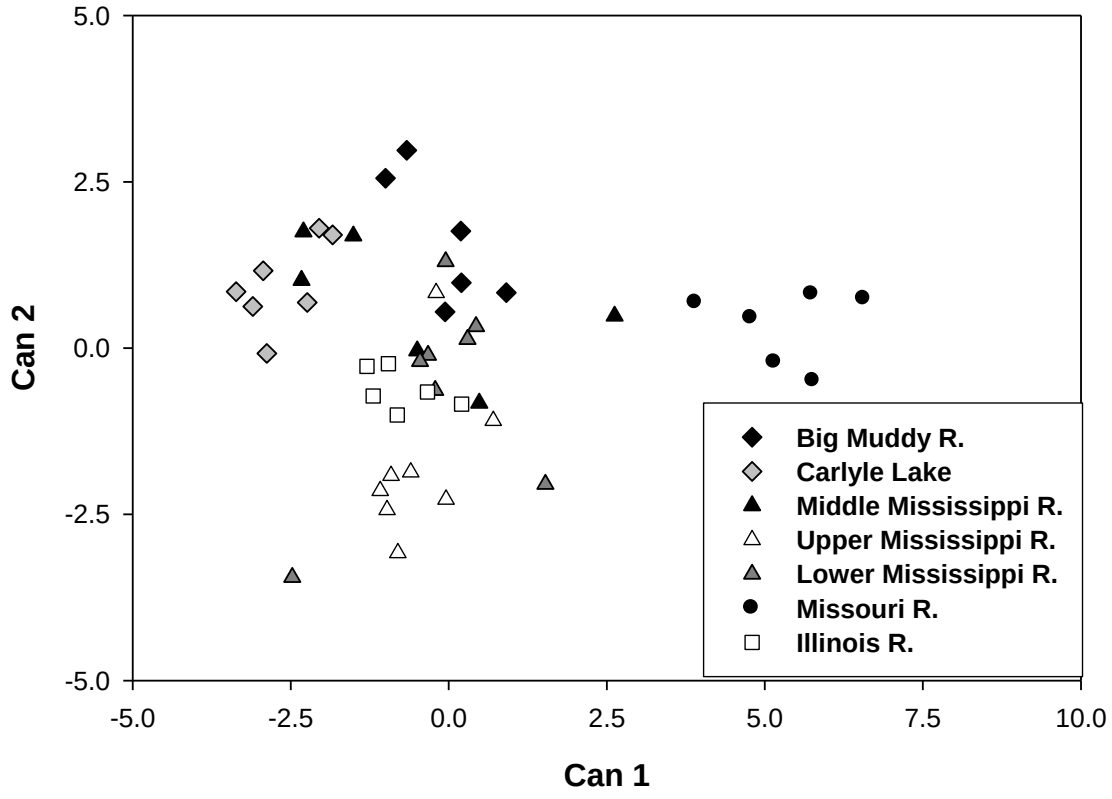

a)
Fin spine Sr:Ca (mmol/mol)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
b)
Otolith Sr:Ca (mmol/mol)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Water Sr:Ca (mmol/mol)
Blue catfish
Channel catfish
Flathead catfish


-4
-5
-6
-7
-8
-9
-10
-11
-7
-6
-5
-4
-3
-2
Otolith δ18O (‰)
Water δ18O (‰)
Blue catfish
Channel catfish
Flathead catfish


Fin spine Sr:Ca (mmol/mol)
Otolith Sr:Ca (mmol/mol)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Blue catfish
Channel catfish
Flathead catfish


a)
Spine Sr:Ca (mmol/mol)
1.0
0.8
0.6
0.4
0.2
0.0
b
cd
a
cd
b
bc
a
d
cd
b)
Otolith Sr:Ca (mmol/mol)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
c
c
a
c
c
c
b
c
c
Big Muddy R.
Carlyle Lake
Fox R.
Illinois R.
Lower Mississippi R.
Middle Mississippi R.
Missouri R.
Swan Lake
Upper Mississippi R.


a)
Otolith $\delta^{18}O$ (‰)
-2
-4
-6
-8
-10
cd
d
bc
bc
c
a
c
b
b)
Otolith $\delta^{13}C$ (‰)
-2
-4
-6
-8
-10
-12
-14
-16
b
b
b
b
b
a
ab
b
Big Muddy R.
Carlyle Lake
Illinois R.
Lower Mississippi R.
Middle Mississippi R.
Missouri R.
Swan Lake
Upper Mississippi R.


5.0
2.5
0.0
-2.5
-5.0
Can 2
-5.0
-2.5
0.0
2.5
5.0
7.5
10.0
Can 1
Big Muddy R.
Carlyle Lake
Middle Mississippi R.
Upper Mississippi R.
Lower Mississippi R.
Missouri R.
Illinois R.